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# EFFECTS OF ELEVATED ATMOSPHERIC CO<sub>2</sub> ON MATURE PLANT RESPONSES IN A NATIVE AUSTRALIAN EUCALYPTUS FOREST (EUCFACE)

Kayla AH Carey  
[kaca6053@colorado.edu](mailto:kaca6053@colorado.edu)

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EFFECTS OF ELEVATED ATMOSPHERIC CO<sub>2</sub> ON MATURE PLANT RESPONSES  
IN A NATIVE AUSTRALIAN *EUCALPYTUS* FOREST (EUCFACE)

By  
Kayla Carey  
Ecology and Evolutionary Biology, University of Colorado at Boulder

Defense Date: March 16, 2016

Thesis Advisor:  
Dr. Barbara Demmig-Adams, Ecology and Evolutionary Biology & Honors Council  
member

Defense Committee:  
Dr. Barbara Demmig-Adams, Ecology and Evolutionary Biology & Honors Council  
member  
Dr. Pieter Johnson, Ecology and Evolutionary Biology & Honors Council member  
Dr. James White, Geological Sciences & Environmental Studies

## **Abstract**

Rising atmospheric carbon dioxide (CO<sub>2</sub>) concentrations are associated with rising global temperatures; these changes alter ecological communities in ways that vary among plant species and environmental conditions. Research on the response of various plant species to elevated CO<sub>2</sub> concentration is needed to predict future implications of climate change on ecosystems. Free-Air-CO<sub>2</sub> Enrichment (FACE) experiments examine the effects of elevated atmospheric CO<sub>2</sub> levels on terrestrial ecosystems by manipulating atmospheric CO<sub>2</sub> concentration of native vegetation.

The present study was the first ever FACE experiment in a mature Australian *Eucalyptus* woodland and simulated an ecosystem exposed to CO<sub>2</sub> levels of 550 parts per million (ppm), which is the projected global atmospheric CO<sub>2</sub> concentration for 2050. Control plots were exposed to un-manipulated atmospheric CO<sub>2</sub> levels of approximately 400 ppm. The influence of elevated atmospheric CO<sub>2</sub> on the ecosystem was assessed by analyzing photosynthesis rates, radial tree growth (trunk circumference and calculated stem-basal area growth), and photosynthetic water-use efficiency (the ratio of photosynthetic CO<sub>2</sub> uptake to transpirational water loss). This approach led to three major findings: elevated CO<sub>2</sub> (1) caused photosynthesis to be modestly (2.7%), albeit significantly, higher, (2) did not have an effect on radial tree growth, and (3) was associated with a significantly lower stomatal conductance and greater photosynthetic water-use efficiency. The results of this study are discussed in the context of plant-species-specific differences in the response to elevated CO<sub>2</sub> and elevated temperatures at the ecosystem level.

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## Introduction

Human activity, such as the combustion of fossil fuels (increases CO<sub>2</sub> emissions into the atmosphere) and deforestation (decreases CO<sub>2</sub> uptake from the atmosphere), has caused a dramatic surge in atmospheric carbon dioxide (CO<sub>2</sub>) levels throughout the past century (Soloman et al., 2008; Ghannoum et al., 2010; Reich & Hobbie, 2013). Since the industrial revolution, global atmospheric CO<sub>2</sub> concentration has increased from about 280 parts per million (ppm) to approximately 400 ppm in 2015 (Ghannoum et al., 2010; NOAA/ESRL, 2015). Current atmospheric CO<sub>2</sub> levels are increasing at a rate of 2.5 ppm per year<sup>-1</sup> and are predicted to reach nearly 550 ppm by 2050 (Kallacrackal & Roby, 2011; Friendlingstein et al., 2014). There is a consensus among scientists that CO<sub>2</sub> is a major greenhouse gas that traps heat in the atmosphere and induces global climate change; these changing environmental conditions are predicted to have observable ecological effects (Solomon et al., 2007; Kennedy & Parker, 2010).

The impact of elevated atmospheric CO<sub>2</sub> concentration on plants varies considerably across terrestrial species, environmental conditions, and duration of exposure (Saxe et al., 1998; Nowak et al., 2004; Körner, 2006; Kallacrackal & Roby, 2011; Ellsworth et al., 2014). One main conclusion has been that the effect of fertilization of plants with CO<sub>2</sub> depends strongly on whether or not plant growth is limited by other environmental constraints, such as water availability, temperature, and mineral nutrition (Saxe et al., 1998; Oren et al., 2001; Reddy et al., 2010). Much attention has also been focused on indirect effects of elevated CO<sub>2</sub> on plant water relations in recognition of the fact that plants lose water when taking up CO<sub>2</sub> into their

leaves and that this water loss is reduced under elevated CO<sub>2</sub> levels (Morgan et al., 2004; Körner, 2006).

## **Hypotheses**

The three main predictions for this study were:

- (1) Elevated CO<sub>2</sub> will not increase the rate of photosynthesis much in a naturally growing mature *Eucalyptus* forest.
- (2) Elevated CO<sub>2</sub> will not increase radial growth in a naturally growing mature *Eucalyptus* forest.
- (3) Plant water-use efficiency will increase and stomatal conductance will decrease in response to elevated CO<sub>2</sub> in a naturally growing mature *Eucalyptus* forest.

## **Background**

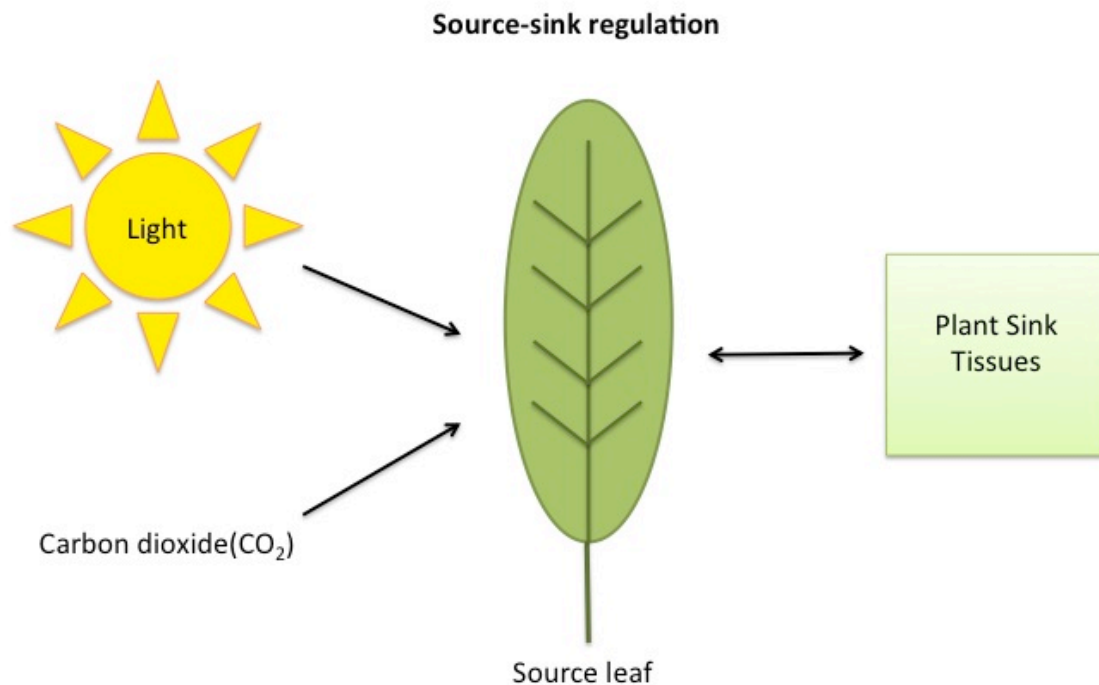
While the concentration of CO<sub>2</sub> in the atmosphere has dramatically fluctuated over geological history (Pearson & Palmer, 2000) it is thought that global CO<sub>2</sub> levels have remained fairly constant and under 500 ppm throughout the past 24 million years (Pearson & Palmer, 2000). However, at the recent rate of increase, atmospheric CO<sub>2</sub> levels are expected to surpass 550 ppm during the current century (Ghannoum et al., 2009; Reddy et al., 2010). Increasing atmospheric CO<sub>2</sub> levels are predicted to influence ecological communities in terms of plant processes and plant composition (Saxe et al., 1998; Hovenden & Williams, 2010; Friedlingstein et al., 2014).

Trees are important primary producers (which convert solar energy to chemical energy) that play a critical role in fuelling global ecosystems (Kallarackal & Roby, 2012). It is important to evaluate tree responses to fluctuating environmental conditions because any sustained changes in primary production would be expected to lead to changes in the stability of a biome (Hovenden & Williams, 2010). However, it is unclear how much sustained change there is in large-scale primary productivity (Reich et al., 2006; Tollefson, 2013). Almost all primary producers use a process called photosynthesis to generate chemical energy (Taiz, 1998). Photosynthesis uses the energy of sunlight to convert CO<sub>2</sub> and H<sub>2</sub>O to sugars used for organic-matter production (Taiz, 1998). The biochemical process of photosynthesis is summarized by the following equation:



Since both light and CO<sub>2</sub> are inputs into the process of photosynthesis, increasing either or both typically initially increases photosynthesis rate up to a saturation point (Ainsworth & Long, 2005; Körner, 2006; Ghannoum et al., 2010). However, the steady rate of photosynthesis is not determined by the substrates of photosynthesis, CO<sub>2</sub> and light, but mainly by the demand from the rest of the plant for photosynthetically produced sugars (Arp, 1991). This regulation by demand is known as source-sink regulation, where the photosynthesizing chloroplast in mature source leaves provides sugars for the sinks of the plant that consist of sugar-consuming growing tissues and storage tissue (Arp, 1991; Krapp & Stitt, 1994; Roitsch, 1999). It has been concluded that only plants that have high growth rates and are not limited by other factors in the environment will respond to elevated atmospheric CO<sub>2</sub> with sustained increases in photosynthesis rates and growth (Körner, 2006). Plants with low intrinsic growth rates and/or exposed to growth-

limiting constraints in the environment will not respond to elevated atmospheric CO<sub>2</sub> with sustained increases in photosynthesis and growth (Körner, 2006). Figure 1 represents the source-sink relationship between photosynthesizing source leaves and sugar-consuming sink tissues of the plant.

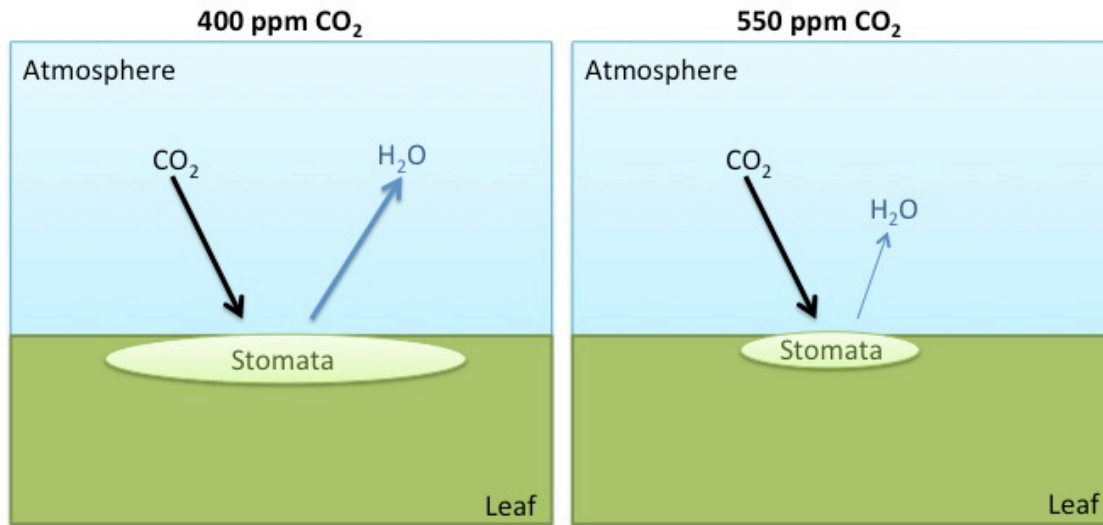


**Figure 1:** Schematic depiction of plant source-sink regulation.

Plants take up CO<sub>2</sub> through the stomata (pore like openings in the leaves), which result in water loss during CO<sub>2</sub> uptake (Taiz, 1998). Exposure to elevated CO<sub>2</sub> has been shown to allow plants to reduce their stomatal opening (stomatal conductance), and thus decrease plant water loss (Figure 2), which increases the ratio of CO<sub>2</sub> fixed to water lost (Sleen et al., 2014). This ratio is known as the plant's photosynthetic water-use efficiency (WUE) (Sleen et al., 2014). Numerous studies examine the indirect effects of



elevated CO<sub>2</sub> on plant water relations, water content, and responses of soil microbial communities (Morgan et al., 2004; Körner, 2006; Nie, 2015).



**Figure 2:** Schematic depiction of the effect of elevated atmospheric CO<sub>2</sub> levels on stomata, leading to reduced stomatal conductance and a reduction in water loss relative to CO<sub>2</sub> uptake.

## Methods

### Study Site

The present study was conducted in the Cumberland Plain Woodland via a Free-Air-CO<sub>2</sub>-Enrichment (EucFACE) experiment located in Richmond, New South Wales, Australia within a flat 35 ha tract of mature *Eucalyptus* open forest (Drake et al., 2015). The over-story vegetation (highest layer of the forest canopy) was dominated by a single *Eucalyptus* species, the forest red gum (*Eucalyptus tereticornis*) (Drake et al., 2015). *E. tereticornis* was chosen as the species on which my measurements focused. Other, less prominent trees and shrubs contributing to the forest layers included White Feather honeymyrtle (*Melaleuca decora*), parramatta wattle (*Acacia parramattensis*), coffee bush

(*Breynia oblongifolia*) and needlebush (*Hakea sericea*) (Drake et al., 2015). The soil was dry and slightly acidic; its texture was categorized as loamy sand to a depth of about 50 to 80 cm, after which the soil transitioned into sandy clay loam (Drake et al., 2015). The coordinates for the first plot (ring 1) in the experiment were Lat. 33°36'59.16"S, Long. 150°44'16.93"E. Figure 3 provides an overview map of the study location.

## **Study Organism**

The tree species, on which the present study focused, was the forest red gum tree (*E. tereticornis*), which is native to Australia (Drake et al., 2015). The forest red gum has a broad distribution, over 3,400 km of latitude, in eastern Australia, occurring from far southern NSW (38° S) to the northern part of the York Peninsula (15° S) (Boland et al., 2006).

## **Experimental Set-Up**

The experiment included a set of fully instrumented controls with six identical 25 m (diameter) circular plots ('rings'), displayed in Figure 4. Plots were connected by a set of glass-fiber vertical pipes that controlled the level of CO<sub>2</sub> throughout the ecosystem (Figure 5). Rings were divided into two categories, with three of the plots at ambient levels of CO<sub>2</sub> (400 ppm) and the remaining three plots exposed to enriched levels of atmospheric CO<sub>2</sub> (550 ppm). Heat from the air vaporized CO<sub>2</sub> before it was released from the pipes at ambient air temperature and injected into the rings in a controlled manner. The control rings with ambient CO<sub>2</sub> levels were randomly selected as rings 2, 3, and 6; rings 1, 4, and 5 were injected with an elevated level of CO<sub>2</sub> from the upwind side. A central computer controlled the CO<sub>2</sub> level of the experiment according to minute-by-

minute meteorology to create constant but different atmospheric CO<sub>2</sub> levels. Trees were numerically labelled clockwise around each ring. The density of the forest was about 600 trees ha<sup>-1</sup>. Open chambers were used to reduce interference with tree growth. (Drake et al., 2015)



**Figure 3.** Overall site map of the Australian *Eucalyptus* Free-Air CO<sub>2</sub> Enrichment experiment; the site included six circular plots that were randomly numbered and assigned as control or experimental rings. The control plots (2, 3, and 6) were set at ambient atmospheric CO<sub>2</sub> levels (400 ppm) and the experimental plots (rings 1, 4, and 5) were manipulated to experience an enriched CO<sub>2</sub> concentration (550 ppm).



**Figure 4.** Photographic image of rings in the EucFACE study site. The site consisted of six identical 25-m carbon-fiber circular rings, three of which were injected with elevated CO<sub>2</sub> (550 ppm), while three other rings were set at ambient CO<sub>2</sub> levels (400 ppm). Plots were all equipped with crane technology to allow researchers to be lifted above the ring for data collection. Photo courtesy of David S. Ellsworth.



**Figure 5.** Photographic image of the piping system in the EucFACE site. Liquid CO<sub>2</sub> was released by a controlled computer system through interconnected vaporizer pipes that ran through the forest. All piping was elevated from the forest floor to prevent interference with the flow of the ecosystem.

## Data Collection

### Rates of Physiological Processes

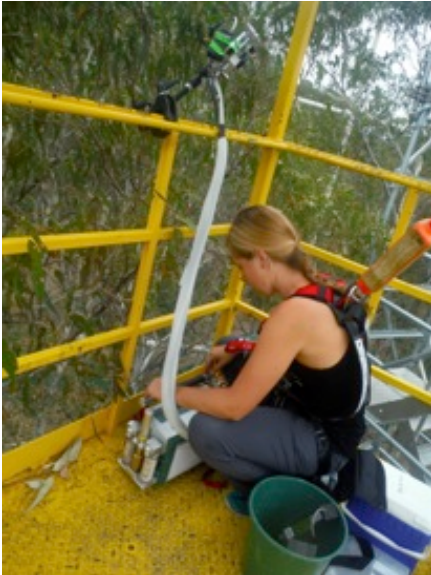
I was lifted in a crane above the rings in order to sample leaves from the uppermost level of the forest canopy. Leaf photosynthesis and stomatal conductance rates were measured using a portable photosynthesis instrument, Li-6400 (Li-COR, Lincoln, Nebraska USA), between 18 November 2014 and 26 November 2014 throughout the hours of 09:00 and 11:00. The system included two independent gas analyzers and measured concentration differences to calculate physiological process rates. The software used in the Li-Cor 6400 for this research was “OPEN v 6.1”. The instrument’s computer allowed for control of environmental conditions within the leaf chamber. The selected physiological parameters for the experiment were:

- Ambient CO<sub>2</sub> Rings (2, 3, and 6)
  - CO<sub>2</sub> Level: 400 ppm
- Elevated CO<sub>2</sub> Rings (1, 4, and 5)
  - CO<sub>2</sub> Level: 550 ppm
- Both sets of rings experienced the same light intensity, temperature, and flow rates
  - Light: 1500  $\mu\text{mol photons/m}^2/\text{s}$
  - Flow: 500  $\mu\text{mol/s}$
  - Temperature: 30°C

While lifted above the ring, I selected an undamaged leaf from the uppermost level of the canopy in each plot. The designated leaf was then placed in the leaf chamber for measurements. An automatic program collected data throughout the sample period.



The console computer recorded measurements every 15 seconds for 20 minutes. Data were then transferred from the machine to a laptop for further analysis.



**Figure 6.** Photographic image of Kayla Carey in EucFACE site crane. Leaves from the upper story canopy in the ring were accessed by crane and placed into the leaf chamber of a Li-Cor 6400. The computer system was used to set the specific parameters for the rings and measure the rate physiological processes in the undamaged leaf.

### **Tree Growth Rate**

The circumference (cm and mm) of the labelled trees was recorded on 11 November 2014 and 12 November 2014 via permanent dendrometer bands (circumference measurement instrument) placed at breast height around the trunk of the trees.

### **Data Analysis**

#### **Physiological Processes**

Average rates of photosynthesis and stomata conductance were calculated for each ring using 80 photosynthetic and conductance measurements per ring. Photosynthesis and conductance rates for all control rings (400 ppm) and all experimental rings (550 ppm), respectively, were averaged to determine mean physiological rates at the two CO<sub>2</sub> levels.

### **Tree Growth Rate**

Dendrometer readings from November were compared to dendrometer site readings from 20 August 2014 (winter). From trunk circumference, cross-sectional area of the tree was calculated as basal area (BA) and used to determine basal area growth in  $\text{cm}^2$ , recorded as basal area incremental increase (BAI) from August 2014 to November 2014. BA of the dendrometer readings from each month was computed by dividing the diameter (cm) by two to obtain the value of the radius (r). BA for each tree was then calculated for both November and August using the equation:

$$BA = \pi r^2$$

BAI ( $\text{cm}^2$ ) was then calculated with the following equation, where  $BA_1$  was the basal area value from August readings and  $BA_2$  was the basal area value from November readings.

$$BAI = BA_2 - BA_1$$

Calculating BAI allowed assessment of growth rates by assessing plant wood production as related to the increasing diameter of growing trees (West, 1980).

### **Photosynthetic water-use efficiency**

Photosynthetic water-use efficiency (WUE), the ratio of carbon uptake rate to transpirational water loss, was calculated for each ring using the following equation:

$$WUE = A_{\text{net}}/T$$

where  $A_{\text{net}}$  was the net rate of photosynthetic carbon uptake and T was the rate of transpiration (as automatically calculated by the Li-Cor 6400). This ratio represents the

trade-off green plants experience between opening stomata for taking up CO<sub>2</sub> during photosynthesis, while losing water through stomata in transpiration.

### **Soil-moisture content**

The EucFACE team regularly monitored moisture content in the soil throughout the site. The Scientific Lead at EucFACE (Dr. David Ellsworth) provided average soil moisture content in ambient and elevated plots throughout the present study (18 to 26 November 2014).

### **Statistical Analysis**

Results were analyzed using the statistical computing software program, R. A Welch's two-sample t-test was conducted on all results to determine statistical differences between ambient and elevated atmospheric CO<sub>2</sub> conditions.

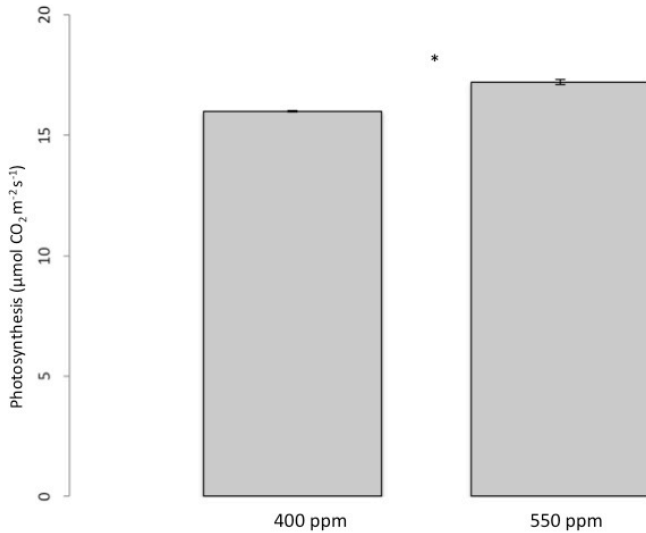
## **Results**

### **Photosynthesis and Growth Rates (Basal Area Increment)**

Net photosynthesis rate of photosynthesis was modestly greater, by 2.76%, (Figure 7) under elevated atmospheric CO<sub>2</sub> (550 ppm; carbon uptake rate of 17.2  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) compared to ambient CO<sub>2</sub> (400 ppm; uptake rate of 16.0  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Figure 8 shows the change in cross-sectional area of tree trunks at breast height in basal area increments (BAI) as a measure of tree growth over a three-month period (from August to November 2014). Mean BAI of trees was not significantly different between ambient (BAI=0.833 cm<sup>2</sup>) and elevated CO<sub>2</sub> treatments (BAI=1.22 cm<sup>2</sup>).

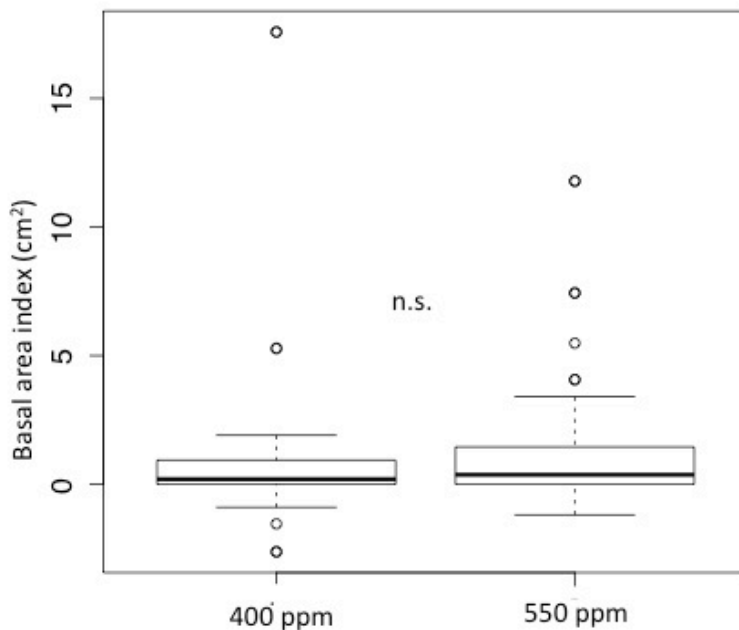


Effect of atmospheric CO<sub>2</sub> concentration on the rate of photosynthesis in *E. tereticornis*



**Figure 7:** Comparison between photosynthetic carbon uptake ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) by leaves in the upper canopy of *E. tereticornis* in a *Eucalyptus* free-air CO<sub>2</sub> enrichment (FACE) experiment. Photosynthesis was measured at 30°C every 15 seconds for 20 minutes in an undamaged leaf at ambient (400 ppm) and elevated (550 ppm) CO<sub>2</sub> conditions. Error bars show standard error of the mean ( $P < 2.2\text{e-}16$ ;  $n=80$ ).

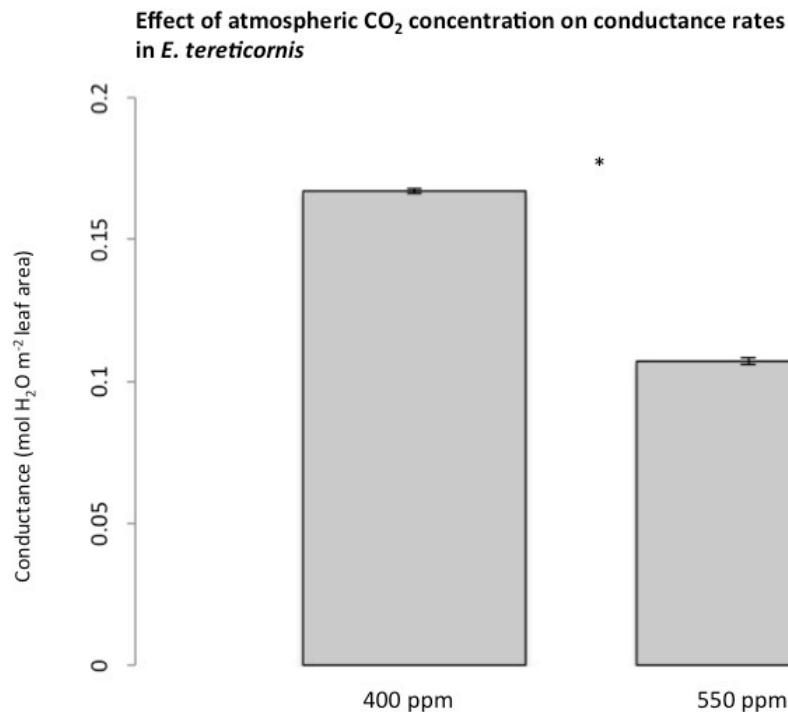
Effect of atmospheric CO<sub>2</sub> concentration on basal area in *E. tereticornis*



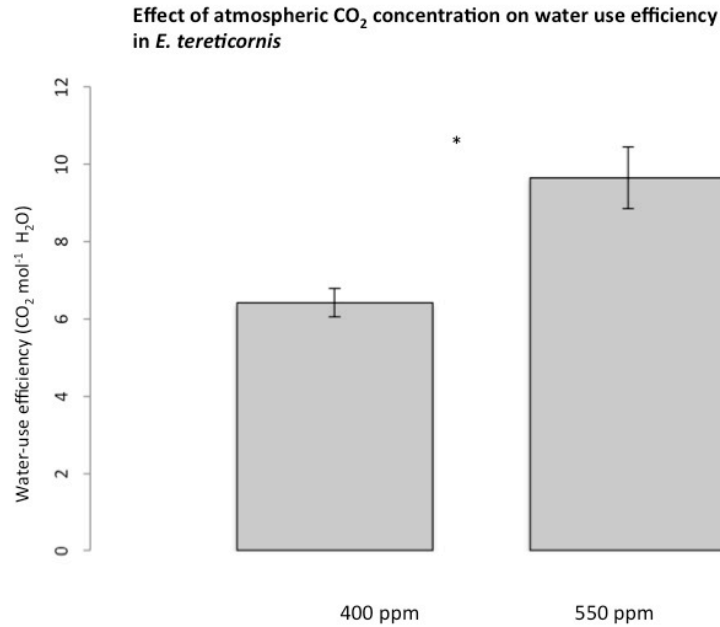
**Figure 8:** Comparison of the growth of basal area index (BAI) of *E. tereticornis* exposed to 400 ppm CO<sub>2</sub> and 550 ppm CO<sub>2</sub>. BAI describes the cross sectional area of the trunk of the tree at breast height. Measurements were taken over of three-month time period in a *Eucalyptus* free-air CO<sub>2</sub> enrichment (EucFACE) experiment. ( $P=0.3325$ ;  $n=162$ )

## Stomatal Conductance & Water-Use Efficiency (WUE)

Stomatal conductance, as a measure of water loss from leaves, in trees exposed to elevated atmospheric CO<sub>2</sub> conditions (550 ppm) was significantly lower than that of trees exposed to ambient CO<sub>2</sub> (400 ppm) (Figure 9). Consequently, photosynthetic water-use efficiency (ratio of photosynthesis rate to transpiration rate) was significantly higher in leaves of trees exposed to elevated versus ambient CO<sub>2</sub> (Figure 10).



**Figure 9:** Comparison of relationship of average leaf stomatal conductance from leaves in the upper canopy of *E. tereticornis* in an open woodland *Eucalyptus* forest exposed to 400 ppm CO<sub>2</sub> vs. 550 ppm CO<sub>2</sub> in a free-air CO<sub>2</sub> enrichment (FACE) experiment. Conductance rates at 30°C were recorded every 15 seconds for 20 minutes in a randomly chosen undamaged leaf. The mean rate of conductance was calculated for each plot and then the average was found for ambient rings (400 ppm) and elevated rings (550 ppm). Error bars show standard error of the mean. ( $P < 2.2 \times 10^{-16}$ ;  $n=80$ )



**Figure 10:** Comparison of photosynthetic the water-use efficiency (WUE, as the ratio of the rates of photosynthetic carbon uptake to transpirational water loss) of leaves in the upper canopy of *E. tereticornis* in an open-woodland *Eucalyptus* forest exposed to 400 ppm CO<sub>2</sub> versus 550 ppm CO<sub>2</sub> in a *Eucalyptus* free-air CO<sub>2</sub> enrichment (EucFACE) experiment. WUE is the ratio of the rate. Error bars display standard error of the mean. (P=0.038; n=80)

### Soil water content

Soil water content of the soil below the *Eucalyptus* trees was greater for trees exposed to elevated versus ambient CO<sub>2</sub> (Table1).

	400 ppm	550 ppm
Average moisture content	2.4%	3.1%

Table 1: Average moisture (H<sub>2</sub>O) content in ambient (400 ppm) and elevated (550 ppm) CO<sub>2</sub> plots during 18 November 2014 to 26 November 2014 at the *Eucalyptus* free-air CO<sub>2</sub> enrichment (EucFACE) experiment.

## **Discussion**

### **Main Trends**

The main findings observed throughout this EucFACE study support the original hypotheses regarding plant responses to elevated levels of atmospheric CO<sub>2</sub>:

- (1) Elevated CO<sub>2</sub> lead to a significant, albeit small, increase in the rate of photosynthesis.
- (2) Elevated CO<sub>2</sub> did not cause a significant increase in radial tree growth.
- (3) Plant water-use efficiency significantly increased in elevated CO<sub>2</sub> along with a significant decrease in stomatal conductance.

### **Photosynthesis and tree growth**

The current study revealed only a minimally greater photosynthesis rate, without a significant difference in apparent tree growth between trees exposed to elevated and ambient atmospheric CO<sub>2</sub> levels. It cannot be excluded that the three-month period over which adult tree growth was monitored in the present study was insufficient to reveal significant differences. Furthermore, the available data on tree trunk circumference do not provide information on any vertical growth. However, it is clear that (i) growth of tree circumference is relatively slow and (ii) photosynthesis rates are rather low with only a minimal difference between CO<sub>2</sub> treatments, which is unlikely to support greater growth rates in the trees exposed to elevated CO<sub>2</sub>. A plant system that responds to elevated atmospheric CO<sub>2</sub> levels with sustained increases in photosynthesis and growth would have the potential to mitigate the effects of increasing atmospheric changes by acting as a greater sink for carbon storage (Ghannoum et al., 2010; Tollefson, 2013; Nie

et al., 2015). The present data suggest that the *Eucalypt* forest studied here is not a significant sink for additional CO<sub>2</sub>.

These results are consistent with findings from other studies on native plant communities where elevated CO<sub>2</sub> did not increase photosynthesis rates or growth in a sustained manner (Ellsworth et al., 2004; Sleen et al., 2014). It is likely that *Eucalypt* growth rates were limited by factor(s) other than CO<sub>2</sub>. On the basis of the available data for the *Eucalypt* forest studied here, it cannot be determined whether this limitation consisted of a limiting factor in the soil (such as nitrogen, phosphorus, or water) or an intrinsic, genetic limitation to growth rate in this specific tree species. It has been reported that Australian soils are frequently limited in phosphorus and water availability (Oren et al., 2001; Tng et al., 2014). Overall, the lack of a response of tree circumference growth to elevated CO<sub>2</sub>, and the only very minor stimulation of photosynthesis rates, are consistent with previous conclusions for many natural ecosystems where growth limitations prevent a lasting increase in photosynthesis rates (Sleen et al., 2014).

Previous research suggests that effects of rising levels of CO<sub>2</sub> may affect plants utilizing different photosynthetic pathways (C<sub>3</sub> vs. C<sub>4</sub>) differentially (Reddy et al, 2010; Ghannoum et al., 2010; Kallarackal & Roby, 2012). C<sub>4</sub> plants concentrate CO<sub>2</sub> internally to prevent fixation of oxygen instead of CO<sub>2</sub> by the CO<sub>2</sub>-fixing enzyme in the process of photorespiration, while C<sub>3</sub> plants do not concentrate CO<sub>2</sub> internally and thus potentially respond more strongly to additional CO<sub>2</sub> in the atmosphere via increased carbon fixation (Ainsworth & Long, 2005; Reddy et al., 2010; Kallarackal & Roby, 2012). Nevertheless, FACE experiments suggest that C<sub>3</sub> plants also undergo photosynthetic acclimation in the form of photosynthetic down-regulation after extended

exposure to elevated CO<sub>2</sub> (Kallarackal & Roby, 2012). Elevated CO<sub>2</sub> would presumably initially lead to increased photosynthesis rates, followed by an adjustment of photosynthesis rates to come into equilibrium with the demand of the plant's sinks for sugars produced in photosynthesis (Körner, 2006). Observations made in the Duke Oak Ridge FACE experiment on the species Sweetgum (*Liquidambar styraciflua*) suggested that elevated CO<sub>2</sub> levels can increase plant productivity at a steady rate for certain periods of time in certain systems until productivity eventually begins to decline again, presumably due to genetic or environmental constraints, such as nitrogen limitation and decrease root volume (Ledford, 2008).

Although increased photosynthetic rates are linked to sustained increases in plant growth rates in some species (Ainsworth & Long, 2005), additional research indicates that elevated CO<sub>2</sub> does not result in increased biomass across all species and environments (Kallarackal & Roby, 2010; Reich & Hobbie, 2013; Sleet et al., 2014). A previous experiment, conducted under water- and phosphorus-limited conditions in a grassland ecosystem, led to similar outcomes as the present study, with no significant stimulation in plant growth under elevated atmospheric CO<sub>2</sub> (Reich & Hobbie, 2013). Ghannoum et al. suggests that various *Eucalyptus* species respond differently under enriched atmospheric CO<sub>2</sub> (2010).

On the other hand, it has recently been pointed out that tropical forests may have particularly high rates of photorespiration due to prevailing high temperatures that favor oxygen fixation and photorespiration over CO<sub>2</sub> fixation, and may therefore respond more strongly to elevated CO<sub>2</sub> than non-tropical forests (Hutley et al., 2011; Cernusak et al., 2013). However, the impact of elevated CO<sub>2</sub> on tropical forests remains largely unknown

(Tollefson, 2013). It would be of interest to compare the results from the present study conducted on forest red gum in New South Wales with results on this species from the northern tropical end of its distribution range in Queensland.

### **Conductance & Water Use Efficiency**

Previous research has shown that increased atmospheric CO<sub>2</sub> levels can lead to improved water-use efficiency and a lower stomatal conductance (Hovenden & Williams, 2010; Kallarackal & Roby, 2012; Sleen et al., 2014). The results from the present EucFACE experiment, with significantly lower stomatal conductance rates in plots with elevated versus ambient CO<sub>2</sub>, are consistent with response trends seen in these past studies. A study on other Australian tree species showed a decline in stomatal conductance in 87.5% of study species exposed to elevated levels of CO<sub>2</sub> (Hovenden & Williams, 2010). Consistent with the greater WUE under elevated versus ambient CO<sub>2</sub>, soil moisture content at the EucFACE site, that was extremely low, was even lower in the plots with ambient versus elevated CO<sub>2</sub>, indicating that plants exposed to elevated CO<sub>2</sub> were not taking up as much water from the soil as the vegetation exposed to ambient CO<sub>2</sub>. These results from the present study are consistent with results from previous studies linking an increase in photosynthetic water-use efficiency to an increase in available soil moisture (Kallarackal & Roby, 2012; Sleen et al., 2014).

Increased WUE has the potential to increase plant tolerance to drought conditions (Tollefson, 2013). Furthermore, a majority of Australian forests are affected by fire, and increased plant water content could lead to a decrease in fire frequency and severity. Hovenden & Williams point out that, conversely, any increases in the populations of fire-stimulating species in response to rising atmospheric CO<sub>2</sub> might cause fires to become

progressively more common and severe (2010). If future drought coincides with higher atmospheric CO<sub>2</sub> concentrations, a higher water-use efficiency of vegetation may confer survival advantages under drought conditions.

### **Other Possible Repercussions on Ecosystem**

Elevated levels of atmospheric CO<sub>2</sub> have the potential to alter plant composition with respect to the carbon to nitrogen ratio of plant biomass, which may affect ecological interactions including plant-herbivore exchanges and thus alter species composition of ecosystems (Reich et al, 2006; Ledford, 2008). As a result of altered source-sink relationships, where leaves may produce more sugars under elevated CO<sub>2</sub> levels than can be consumed by the plant's sinks, carbohydrates may accumulate in the leaves. The resulting down-regulation of photosynthesis would decrease the concentration of nitrogen-containing photosynthetic enzymes in the leaves, thus increasing the carbon to nitrogen (C:N) ratio of the foliage (Drake et al., 1997; Hovenden & Williams, 2010). Changes in plant C:N composition can, in turn, affect organisms that depend on the vegetation as a food source via modification of the nutritional value and also of the toxin profile of the plant because C:N ratios influence metabolic processes (Hovenden & Williams, 2010; Reich et al., 2006). An enhanced level of atmospheric CO<sub>2</sub> thus potentially disrupts processes throughout the interconnected terrestrial biosphere (Ellsworth et al., 2004; Kallarackal & Roby, 2012).

### **Discussion and Future Directions**

While the FACE approach focuses on whole ecosystems in natural conditions, the method does have challenges, including financial and infrastructure limitations (Ledford, 2008). In addition, controlling specific environmental conditions, such as temperature,



may prove to be difficult in an open chamber experiment (Ledford, 2008). It is critical to factor in abiotic environmental interactions that influence plant productivity (temperature, soil moisture, and nutrient availability) as well as biotic factors to reduce the risk of overestimating the impact of elevated atmospheric CO<sub>2</sub> on vegetation.

Elevated atmospheric CO<sub>2</sub> has the potential to alter plant performance by influencing stomata conductance and photosynthetic responses, and thus affecting plant productivity and/or plant water relations. Rising atmospheric CO<sub>2</sub> concentration thus has the potential to impact various aspects of individual plant function and resulting ecosystem processes (Saxe et al, 1997; Hovenden & Williams, 2010; Kallarackal & Roby, 2012; Friendlingstein et al, 2014; Evans et al., 2014), but many questions remain open (Ainsworth & Long, 2005; Körner, 2006; Ledford, 2008). Additional research is needed to derive concrete predictions on the effects of rising atmospheric CO<sub>2</sub> on ecological communities. Long-term, whole-forest studies are crucial for a better understanding of the effects of elevated CO<sub>2</sub> on biochemical relationships and ecological community structure in order to derive recommendations for further research and environmental conservation (Ainsworth & Long, 2005; Ghannoum et al., 2010; Reich & Hobbie, 2013). It is especially important to examine the reactions of both tropical and high-latitude forests due to their key role in the terrestrial carbon cycle (Ledford, 2008; Körner, 2006). A better understanding of potential major carbon sinks under changing environmental conditions is needed to aid in efforts to mitigate global climate change (Ghannoum et al., 2010; Ledford, 2008).

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